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EFFICACY TRIALS FOR KNEE CARTILAGE CHANGE MAY ACHIEVE REASONABLE TREATMENT GOALS IN≤12 MONTHS AND SAMPLE SIZE≤200/ARM

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Purpose: The choice of an efficacy biomarker for cartilage change in a clinical trial for structural treatment of osteoarthritis depends on expected treatment effects, disease progression patterns, and trial length. Previous work has shown that local increases and decreases in cartilage thickness in study participants may occur over short periods of time. Presumably treatment would minimize both thinning and thickening aspects of disease progression, but could also lead to localized or global cartilage growth. Hence simple averaging of cartilage loss across joint compartments or cartilage plates may not efficiently capture the complexity of thickness change, likely to be seen in shorter clinical trials. This study examines different metrics of cartilage thickness change for clinical trials under different trial lengths and treatment effects.
Methods: Estimations of sample size (N) for clinical trials were based on differences in cartilage thickness change observed in coronal MR images (3 Tesla) of 145 women (71 with medial radiographic osteoarthritis (ROA), 74 with no symptoms or ROA) at baseline, 3, 6, 12 and 24 months of an observational trial. Femorotibial cartilage thickness was determined across 5 tibial and 3 femoral subregions in each (medial/lateral) compartment. Sample size per arm (treatment, control, two-sample t-test) was used to measure the efficiency of different clinical trial and biomarker choices. Three treatment effects were considered: disease progression, the mean difference in change between ROA and asymptomatic subjects, is reduced by 50%, and global growth of 2% or 5%. Two trial parameters were explored: trial length (3, 6, 12 or 24 months), and whether a single or multiple visits were included in the statistical analysis. All biomarkers considered were univariate, i.e., they summarized change from multiple regions and visits into a single value per subject. Other biomarker parameters examined: use of thickness change (c) or magnitude (absolute value) of change ($|c|$); choice of regions (all, medial, central or external (C/E) medial regions or C/E medial regions individually or choice dependent on magnitude of change); methods of summarizing over regions and visits (average, maximum or minimum).
Results: Multi-visit metrics had smaller N than single visit metrics. A 3 month visit was not informative; N for 3 month trials were large, N > 325 and was typically more than twice as large as needed at 6 months; and including a 3 month visit did not reduce N in multi-visit metrics. Biomarkers averaging all or just medial regions had smaller N than individual regions or the average of the C/E medial regions. Table 1 reports results for biomarkers that consistently had small N across visits for at least one of the treatment effects. Averages of the regions with the most positive or most negative changes were often the most efficient biomarkers, but how these changes were included depended on treatment scenario. When the treatment slowed cartilage disease progression, trials using $|c|$ had smaller sample sizes ($87 < N < 166$) than using thickness change, c ($N > 168$) regardless of trial length. On the other hand, with global 5% growth, biomarkers based on c were estimated to have much smaller sample sizes ($N< 100$) than when based on $|c|$ ($N > 300$), except when average $|c|$ over all regions in 24 month trials ($N = 58$). The most efficient change biomarkers under 5% global growth were the average of all regions and, perhaps surprisingly, the average of the 4 most negative changes. Biomarkers based on c and $|c|$ are nearly comparable for 2% growth treatment scenario. The average of regions with the most negative changes, c, ($67 < N < 204$) was more efficient than using average of regions with largest magnitude $|c|$ ($140 < N < 212$), but not by much and was slightly worse for a 6 month trial.
Conclusions: Efficacy trials for cartilage change of 6 or 12 months may achieve reasonable treatment goals with sample sizes < 200/arm. Generally the most efficient cartilage thickness biomarkers were those that measured the largest negative changes or the largest changes in absolute value, but the choice between these biomarkers depended on trial length and expected treatment benefit.

Table 1
Sample size estimated for efficacy trials with different treatment effects, trial lengths, and biomarker metrics. Trials included visits of 3, 6, 12 or 24 months up to stated trial length, except 6 & 12 month which is a 12 month trial with no 3 month visit. Averaged regions were all regions, or 4 regions with most positive (Top 4) or most negative (Bottom 4) change

		Average change in			Avg magnitude of change in	
Treatment	Trail length	All	Top 4	Bottom 4	All	Top 4
Partial stop	24 Months	>50000	256	326	95	109
	12 Months	9788	254	450	103	106
	6 & 12 Months	8958	204	415	87	102
	6 Months	9038	310	698	138	143
2% Growth	24 Months	76	927	67	281	212
	12 Months	259	10765	107	158	140
	6 & 12 Months	195	21745	96	144	140
	6 Months	828	1696	204	171	161
5% Growth	24 Months	13	32	21	58	1419
	12 Months	35	197	35	15084	1084
	6 & 12 Months	27	124	31	1123	2410
	6 Months	96	1590	72	647	350

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AGGRECAN FRAGMENTOLOGY – PATTERN OF AGGRECAN FRAGMENTS IN DIFFERENT DISEASES

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Purpose: Aggrecan, the main proteoglycan in cartilage extracellular matrix, is proteolysed in joint injuries and in osteoarthritis. The main proteases digesting aggrecan are the matrix metalloproteases (MMPs) and aggrecanases, but aggrecan can also be cleaved by calpains and cathepsins. The question that we asked was: is there a specific proteolytic aggrecan fragmentation pattern (aggrecan fragmentology) which depends on disease and/or disease development?
Methods: Synovial fluid (SF) from subject groups was pooled: knee healthy reference ($n = 10$; age range = 19-58 years), adult knee injuries ($n = 64$; mean age = 33 years), juvenile knee injuries ($n = 9$; age range = 12-15 years), osteoarthritis (OA, $n = 47$; mean age = 48 years), and juvenile idiopathic arthritis (JIA, $n = 120$; age range = 13-19 years). The total sulphated glycosaminoglycan was analysed in the SF-pools by Alcian blue precipitation, and the values were converted to total aggrecan using an assumption that the aggrecan Mw = 1 500 000 g/mol and that 75% of the Mw comes from the glycosaminoglycan's. Aggrecan fragments were purified from the SF-pools using CsCl density gradient centrifugation collecting the SF-D1 fractions. Purified calf aggrecan (A1D1 fraction from CsCl density gradient centrifugation) was used as full length G1-G3 standards for Western blot quantifications, and total degradation of purified human aggrecan (A1D1 fraction) was used to make ARGS (aggrecanase-1 digest), FFGV (MMP-3 digest) and PGVA (m-calpain digest) standards. The standards and the SF-D1 samples were deglycosylated and visualized by Western blot using anti-aggrecan neoepitope antibodies ARGS, FFGV, PGVA and an anti-aggrecan G3 antibody. Total amount (mean pmol) of ARGS, FFGV, G1-G3, inter globular domain (IGD)-PGVA and chondroitin sulfate region 2 (CS2)-G3 fragments was calculated (using Western blot and standards) for each subject group, and the proportion of fragments (% of aggrecan) was calculated in relation to the total amount of fragments detected in each group.
Results: The total amount aggrecan detected in the SF pools was approximately 2-3 higher in the knee injury groups (adult and juvenile injury) and in the OA group (101 – 129 pmol/ml) compared to the knee healthy reference and the JIA groups (44 and 45 pmol/ml). The distribution of full length aggrecan (G1-G3) and of aggrecanase (ARGS and CS-G3), MMP (FFGV) and calpain (IGD-PGVA) generated fragments was different between patient groups (Fig. 1). Of the total amount of fragments detected, calpain generated PGVA fragments was low in the different patients groups, although the reference group contained 7% PGVA fragments. The adult and juvenile injury groups and the OA group

had a majority of ARGS fragments (70, 61 and 77%, respectively), while the reference and the JIA groups had high proportion of CS2-G3 fragments (53 and 52%, respectively). Estimates of the relative mol-mol proportions of the pathological aggrecanase cleavage in the IGD generating ARGS-fragment and the aggrecanase turnover cut in the CS2 region generating CS2-G3 fragments showed that the ARGS proportion was 27% for the reference group while it was between 69 and 82% in the adult and juvenile injury groups and in OA group. A similar comparison of MMP generated FFGV (a cut in the IGD of aggrecan) and the aggrecanase generated ARGS fragments showed that the FFGV fragments amounted to 32% for the reference group while the proportion was much lower for the adult injury (2.1%), juvenile injury (7.3%) and the OA (1.2%) groups.

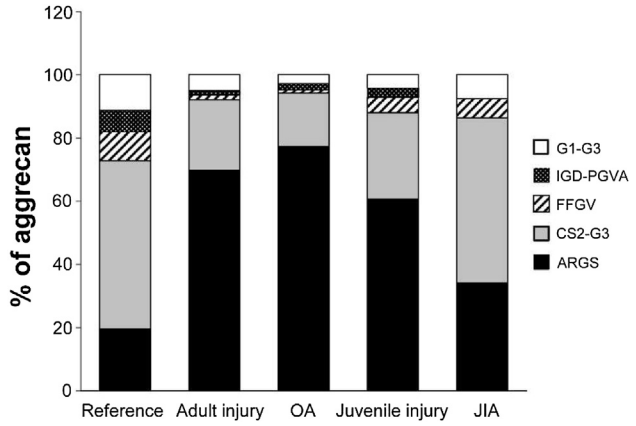

Fig 1. Proportion (mol/mol) of full length aggrecan (G1-G3), aggrecanase generated ARGS and CS2-G3 fragments, MMP generated FFGV fragments and calpain generated IGD-PGVA fragments found in synovial fluid pools of different patient Groups.

Conclusions: The OA, juvenile and adult knee injury groups show similar aggrecan fragmentation patterns, which differ from the fragmentation pattern of JIA and knee healthy reference groups. This suggests that the aggrecan fragmentation patterns are different between different joint diseases. This information supports further understanding of mechanisms of cartilage damage in these conditions, and may aid to distinguish different patient groups.

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RELATIONSHIP BETWEEN CONCOMITANT INJURIES SUSTAINED DURING ACL RUPTURE AND BIOLOGICAL MARKERS OF ARTICULAR CARTILAGE METABOLISM

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Purpose: There is limited information regarding the onset and earliest stages of post-traumatic osteoarthritis (OA), which is commonly associated with rupture of the anterior cruciate ligament (ACL). Consequently, the purpose of this investigation was to examine relationships between concomitant injuries to the tibiofemoral articular cartilage sustained during acute ACL injury with patient-oriented outcomes as well as biochemical markers of type II collagen metabolism and aggrecan degradation, compared to healthy, matched controls.
Methods: Thirty-nine ACL-reconstructed (20 women) and 32 knee healthy control (18 women) subjects matched for age, sex, race, BMI, and activity level were evaluated in this cross-sectional study. Inclusion criteria for injured subjects was: age 14-55yrs, BMI between 18.5-30, Tegner activity score $\geq$ 5, no previous knee pathologies, normal anatomic tibiofemoral alignment, $<$ 2/3 meniscectomy performed at surgery, and $\leq$ grade 3A articular cartilage lesions (based on International Cartilage Repair Society [ICRS] classification). Similar inclusion criteria were utilized for controls with the exception of: no history of knee pain or dysfunction, normal clinical knee examination, and no abnormalities on MRI. Articular cartilage lesions were identified under direct arthroscopic visualization at the time of ACL reconstruction and were documented by one of two sports medicine fellowship-trained orthopaedic surgeons. Injured subjects were classified as low-risk for future OA development if they displayed $\leq$ grade 2 articular cartilage lesions. Injured subjects were classified as high-risk for future OA development if they displayed grade 3A articular cartilage lesions. Synovial fluid samples were obtained from injured subjects immediately prior to surgery, and from controls at a single time point. The mean interval between index injury and surgery date was 70.1 days; range: 18-155 days. Synovial fluid markers of type II collagen synthesis were evaluated by measuring concentrations of procollagen II C-propeptide (CPII) with ELISA (Ibex). Markers of type II collagen degradation were also evaluated with ELISA and included collagen type II cleavage product (C2C; Ibex) and collagen type I and II cleavage product (C1,2C; Ibex). Additionally, the Alanine-Arginine-Glycine-Serine (ARGS) neoepitope was measured as a marker of aggrecan degradation using an electrochemiluminescence in-house immunoassay. Patient oriented outcomes were evaluated in all subjects with the Knee Injury and Osteoarthritis Outcome Score (KOOS). Analysis of Variance was performed for statistical evaluation.
Results: Of the 39 ACL reconstructed individuals, 29 (74%) had articular cartilage injuries that were grade 2 or less, while 10 (26%) had grade 3A articular cartilage injuries. Controlling for sex, BMI, activity level, and time between injury and baseline measurements, there were no significant differences in mean levels of markers of type II collagen metabolism or aggrecan breakdown ($p = 0.48$ and $p = 0.55$, respectively) between risk groups. Associations between ARGS concentration and KOOS subscales of symptoms and pain were found to be significantly different between groups ($p = 0.03$ and $p = 0.01$, respectively). These significant interactions were driven by positively correlated associations between KOOS scores and ARGS concentration for the high risk group, and negatively correlated associations between KOOS scores and ARGS concentration for the low risk group.
Conclusions: In ACL injured subjects with concomitant grade 3A articular cartilage injuries, levels of synovial fluid ARGS were directly associated with improvements in KOOS symptoms and pain. As a secondary analysis of a longitudinal investigation, this study provides preliminary, hypothesis generating data and may not be adequately powered to elucidate true differences in biomarker concentrations between these groups. Nevertheless, our statistically significant findings may suggest the involvement of synovial fluid ARGS in a localized tissue repair response involving an increase in the synthesis of aggrecan following traumatic knee injury.

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CARTILAGE COLLAGEN NEOEPITOPE C2C AND CLINICAL PARAMETERS IN MIDDLE-AGED PATIENTS WITH KNEE PROBLEMS. CORRELATIONS OF URINARY OUTPUT OF C2C WITH CARTILAGE LESIONS, KOOS VALUES AND FUNCTIONAL ABILITIES OF LOWER LIMB

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Purpose: Intensive research in the last decade has demonstrated that protein biomarkers are required for different purposes in early osteoarthritis (OA): to detect OA, to prognose its progression, to assess efficacy of intervention, etc. Although several biomarkers have acquired definite position in the field, none of the biomarkers applied up to now can sufficiently discriminate individual or limited number of patients (Labefer, van Spil, 2013). One of the likely ways to proceed is to investigate neoepitopes. A collagen type II neoepitope C2C was developed for this purpose.
The aims of the study were to test: (i) the biomarker's ability to differentiate between patients with and without knee cartilage lesion, (ii) if there is any correlation between urinary C2C output and clinical status of patients with early knee osteoarthritis, (iii) preferable option to express results (ng/mmol of creatinine or pg/ml of urine).
Material and methods: We investigated 180 knee OA patients (68 male, 112 female) aged 36-62 (mean 50) yrs. For 112 patients the progression of the knee OA during the past 3 years was available. Standardised radiographs of the tibiofemoral(TF) and patello[[Unsupported Character – Codename -]]femoral(PF) joints were assessed. Radiographic progression was defined as: (i)presence of osteophytes and/or joint space narrowing (JSN) in subjects with no previous radiographic OA or (ii)increase in their grade.